RESEARCH NOTE

High Rates of Positive Hemocultures in Children and Teenagers Seropositive for *Trypanosoma cruzi* in the State of Rio Grande do Sul, Brazil

Cloé D Fernandes, Fátima M Tiecher, Daniela D Fernandes, Nádia MP Henriques, Mário Steindel*/+

Laboratório Central de Saúde Pública, Fundação Estadual de Produção e Pesquisa em Saúde, Porto Alegre, RS, Brasil *Departamento de Microbiologia e Parasitologia, CCB, Universidade Federal de Santa Catarina, Campus Trindade, 88010-970 Florianópolis, SC, Brasil

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The infection by *Trypanosoma cruzi*, the aethiological agent of Chagas' disease, is a serious public health problem in most of the Latin American countries. In Brazil, it is estimated that around five million people are infected by *T. cruzi* and 25 million live in areas of risk (JCP Dias 1987 *Parasitol Today 3*: 336-341). In the South region of Brazil, the State of Rio Grande do Sul is the most important endemic area of human Chagas' disease. The infection rate by *T. cruzi* in different municipalities in the southern region of the state varies from 17.6% to 19.6% (G Baruffa & A Alcântara Filho 1985 *Ann Soc belge Med trop 65*: 171-175). Characterization of several *T. cruzi* strains isolated from the wild and domestic transmission cycles in Rio Grande do Sul shows that these parasite populations are highly polymorphic, as demonstrated by isoenzyme and random amplified polymorphic DNA analysis (CD Fernandes et al. 1997 *Mem Inst Oswaldo Cruz 92*: 343-351).

Several serological tests such as indirect immunofluorescence (IFT), indirect hemoaglutination (IHA) or ELISA have been currently used for the detection of *T. cruzi* infection in humans. Parasitological demonstration of *T. cruzi* in the chronic phase of the disease can be affected by some parameters such as parasite population, infection time and the method (hemoculture or xenodiagnosis) used [E Chiari 1992 Diagnostic tests for Chagas' disease, p. 153-164. In S Wendel, Z Brener, ME Camargo & A Rassi (eds), *Chagas Disease (American Trypanosomiasis): its Impact on Transfusion and Clinical Medicine*, ISBT Brazil' 92, São Paulo]. On the other hand, *T. cruzi* bloodstream parasitemia in chronic chagasic patients may be irregular and varies enormously on different regions. This fact was recently confirmed by the detection of circulating *T. cruzi* using the polymerase chain reaction amplification of minicircle kDNA in chagasic patients (B Zingales et al. 1998 *Int J Parasitol* 28: 105-112). In this work, the authors showed that the infection was confirmed in 10%, 59.4%, 44.7% and 96.5% of the seropositive patients from the states of Amazonas, Piauí, Paraíba and Minas Gerais respectively.

In Rio Grande do Sul, the Fundação Estadual de Pesquisa e Saúde uses IFT, IHA and ELISA test together to confirm *T. cruzi* infection in blood donors and patients under clinical suspicion. The aim of this study was to evaluate the bloodstream parasitemia using a hemoculture technique in children and teenagers from Rio Grande do Sul with positive serological tests for *T. cruzi* infection.

From July 1995 to June 1996, 74 individuals with positive serology for *T. cruzi* from 34 municipalities, most of them from the southern region of Rio Grande do Sul were submitted to a single hemoculture for *T. cruzi* isolation. The age of the patients varies from 4 to 20 years and all individuals presented at least two different positive serological tests for *T. cruzi* infection and were not treated. Epidemiological data from each patient was collected using a standard questionnaire.

For the hemoculture, 30 ml of heparinized venous blood was collected from each patient in a vacuntainer system (Vacuum II^0) and the culture was carried out as described by LMC Galvão et al. (1989 *Braz J Med Biol Res 22*: 841-843). Briefly, plasma was immediately separated by centrifugation at 600 x g/30 min, 4ºC, and stored at -20ºC until use. The pellet was resuspended in 15 ml LIT (liver infusion tryptose) and submitted to the same centrifugation protocol and the supernatant was discharged. Immediately after, LIT medium was added to a volume of 30 ml. Following this procedure, the blood was distributed in six tubes (18 x 200 mm) and incubated at 28ºC. The samples were homogeneized weekly and wet smears were examined for the presence of *T. cruzi* after 30, 60

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+Corresponding author. Fax: +55-48-331.9258. E-mail: ccb1mst@ccb.ufsc.br
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and 90 days. The parasites isolated from positive cultures were stored in liquid nitrogen.

From the 74 seropositive patients submitted to a unique hemoculture, 54 (73%) of them were positive. The number of positive tubes per patient varied from 1 to 6 and the most of the hemocultures (94.5%) were found positive at the 60th day. An additional 5.5% of the cultures turned on positive on the 90th day. The remainig 20 seropositive individuals had negative hemoculture results for up to 90 days. A recent study of 52 non-treated chronic chagasic patients from Minas Gerais with ages between 14 and 82 years, shows a positivity of 94% when three repeated hemocultures were performed per patient and blood was cultivated for 120 days (ZMP Luz et al. 1994 *Rev Soc Bras Med Trop 27*: 143-148).

In spite of its low sensitivity and well known side-effects due to vector saliva, xenodiagnosis has been the most frequent method used for parasitological detection of *T. cruzi* in chagasic patients. A comparison of sensitivity of hemoculture and xenodiagnosis in 80 chronic chagasic patients from Bambuí city, shows a positivity of 30% and 55% for xenodiagnosis and hemoculture respectively (E Chiari et al. 1989 *Rev Soc Bras Med Trop 22*: 19-23). On the other hand, in chronic chagasic patients from Mambaí, State of Goiás, hemoculture and xenodiagnosis showed a similar sensitivity [X Córdova et al. 1996 *Rev Soc Bras Med Trop 29* (Suppl. I): 127]. In our study using a single hemoculture, circulating *T. cruzi* was found in 72% of the seropositive children and teenagers from Rio Grande do Sul. Although we did not seek for IgM anti-*T. cruzi* antibody, the high positive rates of hemocultures found in this study may be due in part to early infections. The age of the patients in the study varied from 4 to 20 years and the most of the patients were children. Hemoculture positivity according to age groups and the number of positive tubes per patient are shown in the Table. The municipalities with higher prevalence of positive hemoculture were Porto Alegre (9/12), São Pedro do Sul (5/6), Arroio do Tigre (5/5), Encruzilhada do Sul (4/5), Piratini (4/4), Santa Rosa de Lima, Bagé and Rio Pardo (2/2) and Pinheiro Machado (3/6). Most of the patients from Porto Alegre, mentioned trips to the endemic area during school holidays or during the Army service. Although Porto Alegre is not considered endemic for Chagas' disease, adults of *Panstrongylus megistus* infected by *T. cruzi* have been frequently collected inside the houses by the local inhabitants or by Fundação Nacional de Saúde personel in several localities of the city. Therefore, the possibility of vector transmission there should not be neglected.

Even though the triatomine control campaign has reduced the vectorial transmission of Chagas' disease in Brazil in the recent years [JCP Dias & AC Silveira, 1996 *Rev Soc Bras Med Trop 29* (Suppl. II): 19-21], congenital transmission of Chagas' disease still occurs. The rates of congenital transmission varies in different countries and in general it is accepted that less than 2% of the infants born from infected mothers will be infected [JCP Dias 1992 Epidemiology of Chagas' disease, p. 49-80. In S Wendel, Z Brener, ME Camargo & A Rassi (eds), *Chagas Disease (American Trypanosomiasis): its Impact on Transfusion and Clinical Medicine*, ISBT Brazil' 92, São Paulo]. Moreover, it is well known that *T. cruzi* may be transmitted through infected blood transfusion. None of the positive patients from this study had been submitted to a blood transfusion. In addition, we investigated the presence of anti-*T. cruzi* antibodies in the serum of 26 related mothers. Eleven of them (42.3%) were seropositive and 15 (57.7%) were seronegative.

TABLE

Hemoculture positivity in children and teenagers from the State of Rio Grande do Sul with positive serology for *Trypanosoma cruzi* infection

Age	No. of patients	Positive hemocultures (%)	Positive tubes per patient
4 - 5	2	1 (50%)	2
6 - 8	5	3 (60%)	2 - 3
9 - 11	28	22 (79%)	1 - 6
12 - 14	20	16 (80%)	1 - 3
15 - 17	7	3 (43%)	1 - 2
18 - 20	12	9 (75%)	1 - 5
Total	74	54 (73%)	-

A recent serological survey of Chagas' disease among 7,021 scholars (6 to 14 years) from 27 municipalities of the endemic area in Rio Grande do Sul shows that 1% of them presented anti-*T. cruzi* antibodies in IFT, IHA and ELISA tests (MF Tiecher 1996, *Características Diferenciais entre Métodos Sorológicos na Infecção Chagásica: Estudo em Escolares de Área Endêmica do Rio Grande do Sul, Brasil*, Ms Thesis, UFRGS, Porto Alegre, 63 pp.). Although congenital transmission cannot be discharged, it is likely that the majority of these infections may be due to early vector transmission. The high rate of positive hemocultures found in this study demonstrated a high level of bloodstream parasitemia in seropositive children and teenagers from Rio Grande do Sul and confirms that this technique might be an additional tool for assessing *T. cruzi* parasitemia in chagasic patients.

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